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Biochemistry

A protein that preferentially binds *Drosophila* satellite DNA

(supercoiled DNA/protein–DNA interaction/sequence specific/*Drosophila melanogaster*)

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ABSTRACT **Using a nitrocellulose filter binding assay, we have detected and partially purified a protein from embryos of *Drosophila melanogaster* that preferentially binds to a highly repeated satellite DNA of the same species. Formation of the satellite DNA–protein complex requires physiological conditions of salt and temperature, but once formed, the complex is stable in high salt (1 M NaCl) or at low temperature. Optimal formation of the specific complex also requires the satellite DNA to be in a supertwisted conformation. The protein interacts with a limited region within the 359-base-pair repeated sequence of the satellite DNA.**

Heterochromatin, the most highly condensed region of the chromosomes of *Drosophila melanogaster*, is located primarily near the centromere of each chromosome. These regions are enriched in highly repeated or satellite DNA sequences. While the differential condensation of heterochromatin ultimately depends on the primary sequence of the heterochromatic DNA, other chromosomal constituents must mediate this folding. Therefore, understanding the molecular mechanisms involved in heterochromatin condensation will require a knowledge of both the organization of satellite DNA sequences and proteins that associate with them. Recently a sequence-specific DNA-binding protein has been isolated from eggs of *D. melanogaster* through the use of the nitrocellulose filter binding assay (1). This protein binds specifically to a cloned segment of *Drosophila* DNA that contains ribosomal DNA sequences (1).

We now report the detection and partial purification of a protein isolated from *Drosophila* embryos that binds in a sequence-specific manner to one of the four major satellite DNAs of *D. melanogaster* (1.688 g/cm^3) (2–4). We have used a competitive nitrocellulose filter binding assay to detect proteins that bind preferentially to a hybrid plasmid containing satellite DNA compared with the vector plasmid lacking the satellite sequence. The protein-binding site has been further localized within the 359-base-pair repeating unit of the satellite to a region containing a symmetric DNA sequence.

MATERIALS AND METHODS

Preparation of DNAs. aDm23-24 is a hybrid plasmid of vector pBR322 (from H. Boyer, ref. 5) carrying a single repeating unit (359 base pairs) of the 1.688 g/cm^3 satellite DNA from *D. melanogaster* (6). The monomer unit in aDm23-24 is derived from the cloned region in pDm23, a hybrid plasmid consisting of pSC101 and 6 kilobases (kb) of 1.688 satellite DNA containing 15 tandem monomer units (4). Satellite DNA monomers were purified from pDm23 DNA by cleavage with *Hae* III and isolated by preparative gel electrophoresis; their 3′ ends were extended with oligo(dC) by terminal transferase. The vector pBR322 DNA was cleaved with *Eco*RI and the 3′ ends were extended with oligo(dG). The satellite monomers were annealed with the vector and used to transform *Escherichia coli* HB101 (*hsm, hsr, recA*) (7) to tetracycline resistance. The recombinant plasmid, aDm23-24, prepared in this way, resulted in the reconstruction of *Hae* III sites at either end of the satellite monomer.

aDm23-24 [^{14}C]DNA was prepared from a 100-ml bacterial culture in K medium containing 25 μCi of [^{14}C]thymine [specific activity 46.4 Ci/mol (1.72 × 10^{12} Bq); New England Nuclear]. Covalently closed, circular plasmid DNA was purified from the cleared lysate by a modified procedure of Katz *et al.* (8), followed by centrifugation twice in ethidium bromide/CsCl gradients (9). After removal of ethidium by *n*-butanol extraction, the DNA was dialyzed into buffer A (10 mM Tris·HCl, pH 7.9/50 mM KCl/0.1 mM Na_3EDTA). Similarly, pBR322 [^{3}H]DNA was purified from 100 ml of bacterial culture in K medium containing 100 μCi of [^{3}H]thymidine (specific activity 55.2 Ci/mmol; New England Nuclear) and 20 mg of thiamine. pBR322 [^{3}H]DNA and aDm23-24 [^{14}C]DNA have specific activities of 6.4 × 10^3 and 4.6 × 10^3 cpm/μg, respectively.

In vivo ^{32}P-labeled aDm23-24 DNA was purified from bacteria grown in 75 ml of low phosphate medium (0.3 mM phosphate) with 3 mCi of carrier-free orthophosphoric acid (ICN) by the procedure described for aDm23-24 [^{14}C]DNA. aDm23-24 [^{32}P]DNA thus isolated has a specific activity of 1.2 × 10^5 cpm/μg.

Nitrocellulose Membrane Filter Assay. The tight binding of proteins to plasmid DNAs was assayed by a modification of the nitrocellulose membrane filtration procedure (10). Nitrocellulose filters (Schleicher and Schuell, BA85; 13 mm diameter, 0.45 μm pore size) were soaked in water for at least 6 hr before use. In each assay, usually 0.14 μg of aDm23-24 [^{14}C]DNA and 0.24 μg of pBR322 [^{3}H]DNA in 60 μl of buffer B (20 mM Tris·HCl, pH 7.9/5 mM sodium phosphate/7 mM $MgCl_2$/60 mM NaCl/0.1 mM Na_3EDTA) were incubated with 6 μl of the protein solution to be assayed. Incubations were performed at 30 or 37°C for 50 min. At the end of the reaction, 1 ml of buffer W (1 M NaCl/10 mM Tris·HCl, pH 7.9/10 mM $MgCl_2$) was added and the solution was then filtered through a nitrocellulose membrane filter that had first been washed with 1.5 ml of buffer W. The filtration rate was ≈3 ml/min. After filtration, the nitrocellulose filter was dried and radioactivity was measured in a Beckman liquid scintillation counter LS-250. The counts from each isotope were calculated after measuring the channel overlap with pure ^{3}H or ^{14}C standards.

Filter assays can be performed under conditions in which the retention is sensitive to the protein concentration by dilution of the protein sample with a solution containing 0.1 mg of bovine serum albumin per ml, 10 mM Tris·HCl, 10 mM sodium phosphate (pH 7.5), 50 mM NaCl, 0.1 mM Na_3EDTA, and 10% glycerol. By this assay, 1 unit of binding protein activity is defined as the amount of protein necessary to retain 50 ng of the aDm23-24 [^{14}C]DNA on the filter. The ratio of aDm23-24 [^{14}C]DNA to pBR322 [^{3}H]DNA bound to the filter measures the specificity of the protein sample. The total amount of

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Abbreviations: $NaDodSO_4$, sodium dodecyl sulfate; kb, kilobase.

plasmid DNA bound to the filter measures the overall efficiency of the reaction, including complex formation and its retention on the filter.

Purification of Satellite Specific DNA-Binding Protein. Embryos from *D. melanogaster* (Oregon R) of average age 1 hr were collected, washed, dechorionated, and homogenized as described (6), except that the homogenization buffer was 0.25 M sucrose/30 mM Tris·HCl, pH 7.9/20 mM NaCl/2 mM Na_3EDTA/1 mM $CaCl_2$. The homogenate of the early embryos was first centrifuged at 3000 × *g* for 10 min to remove nuclei and then at 12,000 × *g* for 20 min to remove mitochondria and some polysomal material. Polymin P (pH 7.9) (polyethyleneimine, BRL, Inc. Rockville, MD) was added to the supernatant to a final concentration of 0.4%. The resulting pellet from Polymin P precipitation was extracted with 1 M NaCl/50 mM Tris·HCl, pH 7.7. After the insoluble material in this extraction mixture was removed by centrifugation, solid ammonium sulfate was added to 70% saturation. The precipitate was collected by centrifugation and resuspended in buffer P [15 mM sodium phosphate, pH 7.5/50 mM NaCl/10% (vol/vol) glycerol/0.1 mM Na_3EDTA/0.1 mM dithiothreitol]. This was then loaded on a phosphocellulose P-11 column. After loading, the column was washed with 2 column volumes of buffer P, and the proteins were eluted with a linear gradient from 0.6 to 1.6 M NaCl in buffer P. The fractions with specific binding activity eluted at ≈1.5 M NaCl and were pooled and dialyzed against 70% ammonium sulfate (in buffer P). The precipitate, collected by centrifugation, was resuspended in 0.5 ml of 20 mM sodium phosphate, pH 7.5/50 mM NaCl/0.1 mM EDTA/0.1 mM dithiothreitol/50% (vol/vol) glycerol and stored at −20°C. This fraction, which has a protein concentration of 2 mg/ml and a specific activity of 1.2×10^4 units/mg, was used in all the experiments presented in the *Results*. This fraction contains ≈0.1% of the proteins in the starting material; there are at least six major polypeptides and several minor ones, as revealed by sodium dodecyl sulfate ($NaDodSO_4$)/polyacrylamide gel electrophoresis. A comparison of the specific activities of the final fraction and the crude extract gives an estimate of 150-fold purification of this binding activity. However, the binding assay of the crude extract is highly unreliable because of the presence of inhibitors that interfere with the assay. For instance, the potent nicking-closing activity in the crude extract would relax the supercoiled DNA substrates used in the binding assays and therefore diminish the binding specificity. (See *Results*).

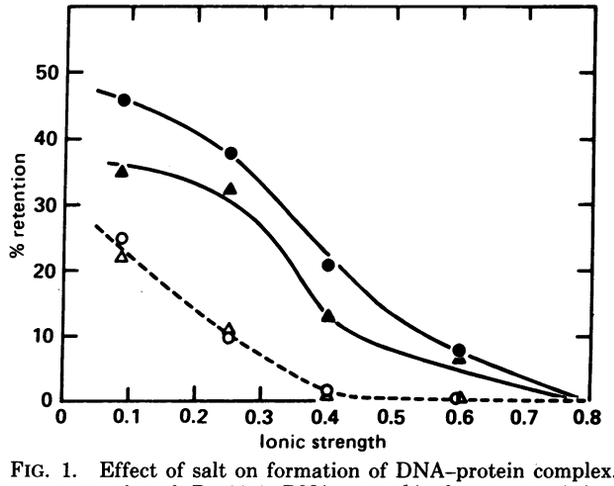

FIG. 1. Effect of salt on formation of DNA–protein complex. Percent retention of aDm23-24 DNA assayed in the presence (●) or absence (▲) of 10 mM Mg^{2+}; vector DNA with (○) and without (△) 10 mM Mg^{2+}. Amount of binding protein used per assay was 1.6 units. Ionic strengths give the concentration of NaCl present during complex formation. After incubation at 30°C for 50 min, the ionic strength of all reactions was raised to 1 M NaCl by addition of 1 ml of buffer W and then the mixture was filtered. The concentration of Mg^{2+} is not included in the calculation of ionic strengths. When bovine serum albumin (10 μg/ml) was used instead of the binding protein, the retention of both DNAs was about 5%. This background retention was subtracted from all the data points. At salt concentration higher than 0.8 M, the percent retention was that of the background.

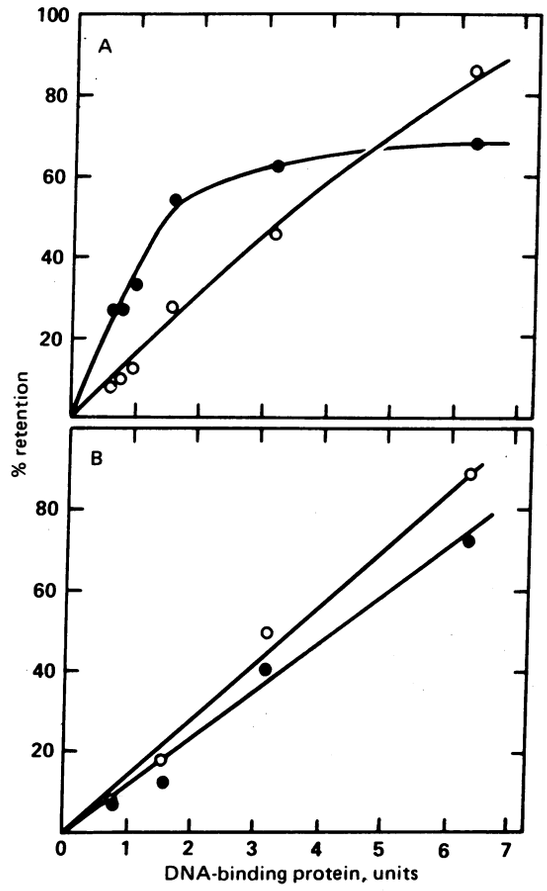

FIG. 2. Specific DNA binding requires DNA in a supercoiled conformation. Percent retention of aDm23-24 DNA (●) and vector DNA (○). DNAs are either in native supercoiled conformation (*A*) or in a nicked relaxed conformation (*B*). DNAs with about one nick per molecule were prepared by pancreatic DNase I treatment in the presence of saturating amount of ethidium bromide as described (11). Background retention is subtracted from all the data points (see legend of Fig. 1).

RESULTS

Ionic Strength and Temperature Are Critical for Specific Complex Formation. In assaying crude embryo extracts for satellite DNA-binding activity, we found that the DNA–protein complex was stable in high ionic strength. Our current assay procedure, therefore, involves the formation of the DNA–protein complex at physiological ionic strengths followed by dilution into 1 M NaCl and filtration through nitrocellulose membrane filters. This procedure markedly reduces background from other weaker protein–DNA interactions that interfere with the assay. While the tight complex is stable in 1 M NaCl, its formation is dependent upon ionic strength (Fig. 1).

At physiological ionic strength (buffer B, 0.1 M salt), plasmid DNA containing the satellite sequence is about twice as efficient as the vector plasmid in forming a DNA–protein complex. The presence of Mg^{2+} increases the formation of the specific com-

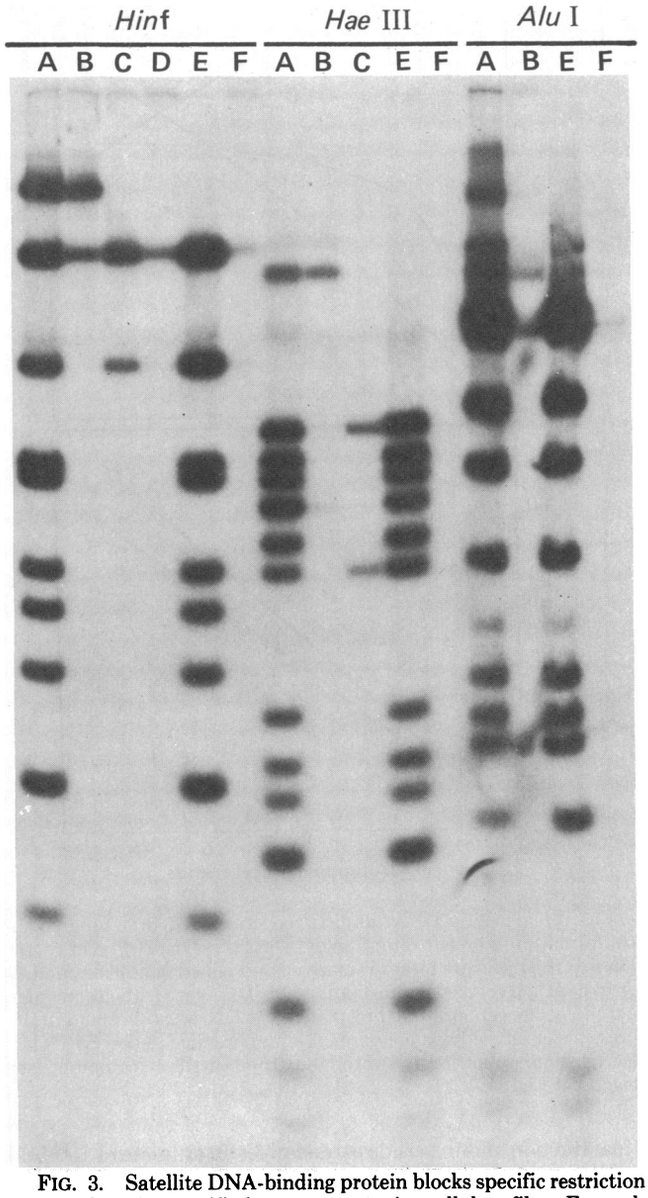

FIG. 3. Satellite DNA-binding protein blocks specific restriction sites and retains specific fragments on a nitrocellulose filter. For each restriction enzyme, slots E and F are controls showing the restriction fragments produced from aDm23-24 DNA (E) and the extent to which these fragments will bind to nitrocellulose in the absence of the DNA-binding protein (F). Slots A, restriction fragments produced in the presence of small amounts of the DNA-binding protein; slots B, those fragments that are retained on the nitrocellulose filter by the binding protein. Slots C in the *Hinf* and *Hae* III experiments show that those DNA fragments retained on the filter are partial digestion products and can be further cleaved after the binding protein is removed. Slot D shows that only a small fraction of the *Hinf* restriction fragments will bind to the filter if the DNA-binding protein is added to the DNA after being cleaved to linear fragments. In a typical experiment, uniformly ^{32}P-labeled, supercoiled aDm23-24 DNA (1.23 μg) was incubated in 106 μl of buffer B with 31 units of satellite binding protein at 30°C for 1 hr. An excess of restriction enzyme was added and incubation was continued at 30°C for another 30 min. An aliquot of the mixture containing 0.23 μg of digested DNA was mixed with an equal volume of 20 mM Tris·HCl, pH 7.7/10 mM NaCl/0.4% $NaDodSO_4$/13 mM Na_3EDTA/1 mg of proteinase K per ml. This aliquot was incubated at 37°C for 30 min and prepared for gel electrophoresis (slots A). The remaining mixture, containing 1 μg of the digested DNA, was diluted in 1 ml of buffer W and filtered. To elute the bound DNA fragments off the membrane filter, we soaked the filter in 200 μl of 20 mM Tris·HCl, pH 7.7/10 mM NaCl/1 mM EDTA/0.3% $NaDodSO_4$ at 37°C for at least 3 hr. The eluate was removed and the filter was further washed with another 50 μl of the same eluting buffer.

plex with aDm23-24 DNA, but has little effect on binding to the vector alone. While an increase in ionic strength will decrease the efficiency of complex formation, it increases the specificity of DNA binding markedly. In a buffer containing 0.4 M NaCl and 10 mM $MgCl_2$, tight complex formation with aDm23-24 DNA is about 10 times more efficient than with the vector pBR322 DNA. When the ionic strength is higher than 0.8, no tight complex is formed. This shows that the addition of 1 M NaCl at the end of each assay is sufficient to stop the reaction.

In addition to ionic strength, incubation temperature is also critical. Lowering the temperature decreases the efficiency and the specificity of complex formation. For instance, at 0°C, the complex formation was decreased to about 1/8th that at 30 or 37°C and very little specificity was observed (data not shown). Therefore, complex formation is favored at lower ionic strength and higher temperature. Once formed, it is stable to high salt or low temperature or both.

Preferential Binding of Satellite DNA Requires a Supercoiled Conformation. Under optimal conditions, the specificity of satellite DNA binding *in vitro* still depends on the conformation of DNA. Fig. 2*A* shows the retention of vector [^{3}H]DNA and aDm23-24 [^{14}C]DNA, both in supercoiled forms, as a function of the addition of the partially purified satellite DNA-binding protein. The binding curve is linear for the vector and hyperbolic for aDm23-24 DNA, suggesting there might be more than one kind of binding site on aDm23-24 DNA. With lower amounts of added satellite DNA-binding protein, the complex formation with aDm23-24 DNA is more efficient than that with the vector DNA. With increasing amounts of the DNA-binding proteins, the specificity decreases. This might be due to the association of satellite DNA-binding protein or other less specific DNA-binding proteins to the nonspecific sites after the specific ones are saturated. In any case, it is necessary to perform the filter binding assays under conditions where retention is less than 50% in order to obtain meaningful linear assay results.

With plasmid DNAs with a single-chain scission per molecule as substrates, the binding curves are both linear and nearly identical (Fig. 2*B*). Similar results were obtained with plasmid DNAs that have been relaxed by the nicking-closing enzyme activity from *Drosophila*. Therefore, under our assaying condition, supercoiling of DNA is prerequisite for the formation of a specific complex.

DNA-Binding Protein Protects Specific Restriction Sites. To locate the specific binding site on aDm23-24 DNA, we investigated the effect of DNA-binding protein on restriction enzyme cleavage. Supercoiled aDm23-24 DNA, uniformly labeled with ^{32}P *in vivo*, was complexed with protein under physiological conditions, cleaved with restriction enzymes, and analyzed by gel electrophoresis. The restriction fragments that

To the combined eluate 25 μl of 4 M ammonium acetate, 36 μg of tRNA, and 750 μl of ethanol was added. The mixture was chilled and pelleted. The pellet was washed with 1 ml of 80% ethanol, dried under reduced pressure, and resuspended in 10 mM Tris·HCl, pH 7.7/50 mM NaCl/0.1 mM EDTA. Half of the material was cleaved with the same restriction enzyme used before (slots C); the remaining half was prepared for gel electrophoresis (slots B). The control experiments were done similarly with 20 μg of bovine serum albumin per ml substituted for the binding protein (slots E and F). All the samples were finally made 0.3% in $NaDodSO_4$, 5% in sucrose, and 0.02% in bromophenol blue and xylene cyanol FF before being loaded on a 6% polyacrylamide gel. The gel was run in a Tris acetate buffer system (12) at a voltage gradient of ≈3 V/cm. After the bromophenol blue dye reached the bottom of the gel, electrophoresis was terminated and the gel was radioautographed at 4°C.

could be retained on the nitrocellulose filter by the binding protein were also determined.

Fig. 3 shows that binding of the protein to aDm23-24 DNA blocked specific restriction sites only in the satellite region, resulting in a partial restriction digest. Furthermore, after cleavage, only those fragments containing satellite DNA were retained on the nitrocellulose filter. For example, *Hinf* digestion of aDm23-24 DNA in the presence of the protein (Fig. 3, *Hinf*-A) revealed a DNA fragment 1.95 kb long which was not present in the absence of the protein (Fig. 3, *Hinf*-E). This fragment resulted from the failure of *Hinf* to cleave at its restriction site within the satellite repeat (see Fig. 4A). This partial product consists of a combination of the two largest *Hinf* fragments of aDm23-24 DNA (1.21 and 0.74 kb), each of which contains satellite sequences. When this restriction digest was passed through a nitrocellulose filter, only the partial product (1.95 kb) and the larger satellite-containing fragment (1.21 kb) were retained. Vector fragments and the 0.74-kb fragment carrying the right half of the satellite repeat were not retained. The DNA fragments bound to nitrocellulose are shown schematically in Fig. 4A. After removal of the DNA-binding protein by $NaDodSO_4$, it is possible to cleave the 1.95-kb fragment into the 1.21- and 0.74-kb components (Fig. 3, *Hinf*-C). A control experiment was performed in which the plasmid was cleaved with *Hinf* and then the DNA-binding protein was added. Fig. 3 *Hinf*-D shows that only a small amount of the 1.21-kb fragment was retained on a nitrocellulose filter compared with *Hinf*-B, in which the complex was formed with supercoiled DNA. This supports our previous conclusions that efficient complex formation requires supercoiled DNA. Nevertheless, one can see some specificity even in the binding of these linear fragments. These experiments show further, that once the DNA protein complex is formed, it is stable even if the DNA is converted to a linear form.

Digestion of the DNA–protein complex with *Hae* III shows that specific *Hae* III sites are also blocked. There are *Hae* III sites at either end of the satellite sequence in aDm23-24 DNA (Fig. 4B). In the presence of the DNA-binding protein, the *Hae* III site at the left end of the satellite region is blocked, resulting in the long partial digestion product present in Fig. 3 *Hae* III-A. Only this partial product was retained on the nitrocellulose filter (Fig. 3, *Hae* III-B). When the DNA-binding protein is removed and this fragment is further cleaved with *Hae* III, one now observes the free satellite monomer (0.36 kb) and the largest *Hae* III fragment (0.59 kb) from the vector (Fig. 3, *Hae* III-C and *Hae* III-E). This large *Hae* III fragment is not, however, immediately adjacent to the satellite region, as is shown by the restriction map (Fig. 4B). Apparently this partial digestion product results from blocking not only the *Hae* III site in the satellite region, but also another *Hae* III site 20 base pairs to the left within the vector. The small fragment released upon cleavage of the long partial product would not be detectable on the gel shown in Fig. 3 *Hae* III-C. These results again demonstrate specific binding of the protein preferentially to the left side of the satellite repeat.

Digestion of the plasmid–protein complex with *Alu* I shows that the *Alu* I site on the left half of the repeat is partially blocked but the *Alu* I site on the right is not blocked (Fig. 4C). Two major *Alu* I restriction fragments are retained on nitrocellulose (Fig. 3, *Alu* I-B). The 0.84-kb fragment corresponds in size with a plasmid fragment that includes the left end of the satellite region, while the larger 1.0-kb fragment is most likely a partial product resulting from failure to cleave at the *Alu* I site (Fig. 4C). From the effect of the DNA-binding protein on these restriction nuclease digestions, it seems to interact with aDm23-24 DNA specifically in the region of the satellite insert. Moreover, the 117-base-pair region between the left end of the satellite region and the first *Alu* I site seems to be most critical for the retention on nitrocellulose by the binding protein. Fragments of satellite DNA carrying the rest of the satellite repeat unit are not retained on the nitrocellulose by the binding protein.

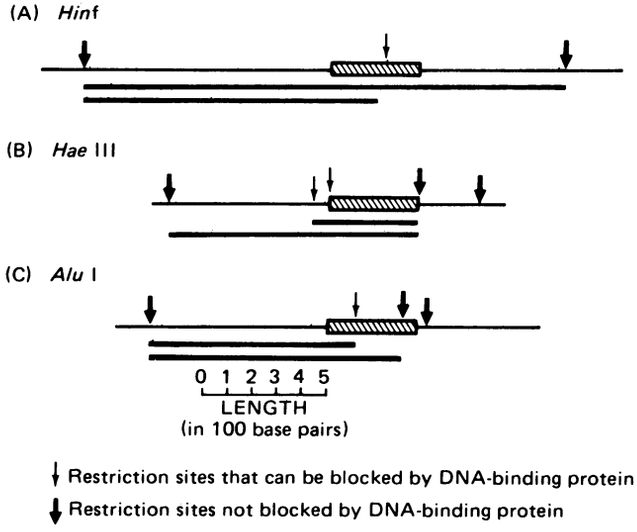

FIG. 4. A restriction map of aDm23-24 DNA showing the blocked restriction sites and fragments retained on the nitrocellulose filter by the satellite binding protein. Shaded bars, cloned *Hae* III monomer of satellite DNA (1.688 g/cm^3); thin lines, the vector part. Thin arrows, restriction sites which are blocked by the binding protein. Thick lines under each map give the fragments that are retained on the filter. The position of these restriction sites were determined from the nucleotide sequence of pBR322 DNA (13).

DISCUSSION

We have partially purified a DNA-binding protein from young embryos of *D. melanogaster* that shows preferential affinity for satellite DNA. We chose as early an embryonic stage as possible because such embryos should be enriched for chromosomal proteins needed during the early, rapid nuclear divisions and because of the success of others at detecting DNA-binding proteins specific for other DNA sequences (1, 14). Our attempts to detect specific satellite binding in proteins extracted from isolated nuclei, from chromatin, or from tissue culture cells have been unsuccessful. This failure is likely due to the very tight association between the binding protein and satellite DNA described above. Satellite specific binding proteins are also not detectable in extracts of late embryos presumably because the proteins associate with the satellite DNA in the newly replicated nuclei.

The optimal conditions for formation of the DNA–protein complex are physiological ionic strength and physiological temperatures. A superhelical conformation of the DNA also enhances the formation of the specific satellite complex. Once formed, the tight complex is stable in high salt, at low temperature, and to relaxation or linearization of the DNA. This kind of irreversibility suggests a significant conformational change in either the DNA or protein upon complex formation. The specificity of the satellite binding protein for supercoiled DNA is reminiscent of the affinity of single-strand DNA-binding proteins for A·T-rich regions in supercoiled DNAs (15, 16). Several experiments, however, demonstrate that the satellite specific DNA-binding protein is not such a protein. First, in a competitive nitrocellulose binding experiment with ϕX174 single-stranded [^{3}H]DNA and aDm23-24 duplex [^{14}C]DNA,

specific binding of the protein to ϕX174 DNA was not observed. Second, we purified two single-strand DNA-binding protein fractions from embryo extracts by DNA–cellulose affinity columns as described (17). Neither fraction showed specific binding of aDm23-24 DNA in our standard assay. Furthermore, the stimulation of specific satellite binding by Mg^{2+} and the stability of the complex in 1 M NaCl distinguish this DNA-binding protein from single-strand DNA-binding proteins. The lack of specificity of the DNA-binding protein for single-stranded DNA and the stimulation of specific binding by Mg^{2+} suggest that the protein recognizes DNA in its native duplex state. The specificity for supercoiled DNA, on the other hand, suggests that the protein may unwind the DNA upon binding.

Restriction nuclease cleavage of the DNA–protein complex indicates that the protein binds most strongly to the 117 base pairs between the *Hae* III and *Alu* I sites at the left end of the satellite region. DNA fragments containing this region bind to nitrocellulose filters, while fragments containing the remainder of the satellite region do not (Fig. 4). Binding to such a limited region at first appears in conflict with the 2-fold specificity observed in the nitrocellulose binding assay. However, since the region essential for nitrocellulose binding is only 1/40th of the entire plasmid, a 2-fold specificity for the plasmid represents at least an 80-fold specificity for the satellite region. This degree of specificity is clearly a minimal estimate since the proteins in our fraction have a general affinity for DNA. Any nonspecific binding to the bulk of the plasmid DNA obscures the true affinity for the satellite region. This specificity is best revealed by analysis of the restriction fragments that are retained on a nitrocellulose filter, where the relative affinity of the proteins for each fragment is displayed. The 359-base-pair monomer released by *Hae* III digestion is not retained on the filter. This result and the fact that the left *Hae* III site is strongly blocked by the protein suggest that an intact *Hae* III site is important for sequence recognition and binding. Sequence analysis of the satellite insert in aDm23-24 DNA indicates a highly symmetric arrangement of nucleotides at the left end near the *Hae* III site (unpublished data). The center of the symmetry contains six contiguous G·C base pairs, whereas the symmetric nucleotides on either side form a rather A·T-rich region. It will be interesting to determine whether this dyad symmetry is involved in the protein–nucleic acid interaction, as has been found for many prokaryotic sequence-specific proteins—for example, *lac* operator (18); cyclic AMP receptor protein (19); and *trp* operator (20).

In addition to the satellite specific binding, this partially purified protein has a high affinity for nucleic acid in general. During the purification it elutes from phosphocellulose at an unusually high ionic strength (1.5 M NaCl). This general affinity for DNA hampers the detection of its sequence specificity by raising the background as described above, thus requiring the use of a competitive filter binding assay (Fig. 1). This assay is very sensitive and can easily discriminate between proteins showing specificity and those without specificity such as the four core histones (H2A, H2B, H3, and H4) or H1, which retain both cloned satellite and vector DNA with equal efficiency (data not shown). Further purification steps have suggested that at least some of the nonspecific binding is due to the presence of other contaminating DNA-binding proteins.

The specificity of the satellite binding protein may be higher *in vivo* than that displayed in our *in vitro* assay. For example, the association of the bulk of the DNA with histones might inhibit nonspecific interactions, while the presence of many tandem copies of the satellite sequence found in the chromosome may facilitate specific satellite binding. On the other hand, the general affinity of a sequence specific protein for DNA may be crucial for its biological function (21). A protein specific for heterochromatin but with a general affinity for DNA could mediate such phenomena as dosage compensation (22) or position effect variegation (23, 24). We have already noted that restriction sites adjacent to satellite sequences are blocked upon complex formation. Since the 1.688 g/cm^3 satellite DNA has been localized primarily to the X and Y chromosomes (25), this specific binding protein could be involved in germ-line function such as meiotic pairing (26) or other postmeiotic processes (27). Further characterization of the interaction of this protein with satellite DNA and with other chromosomal proteins should reveal its role in heterochromatin structure and function.

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